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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 16781/276/BEDL

In re patent application of Daniel CAPUT et al.
Serial No. 07/920,519
Filed: July 28, 1992
For: URATE OXIDASE ACTIVITY PROTEIN, RECOMBINANT GENE CODING THEREFOR, EXPRESSION VECTOR, MICROORGANISMS AND TRANSFORMED CELLS

Allowed: May 31, 1994
Batch: B21
Group Art Unit: 1814
Examiner: D. Schmickel

SUBMISSION OF FORMAL DRAWINGS

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicants submit herewith fifteen (15) sheets of formal drawings for this case.

Respectfully submitted,

August 31, 1994
Date

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07/920,519

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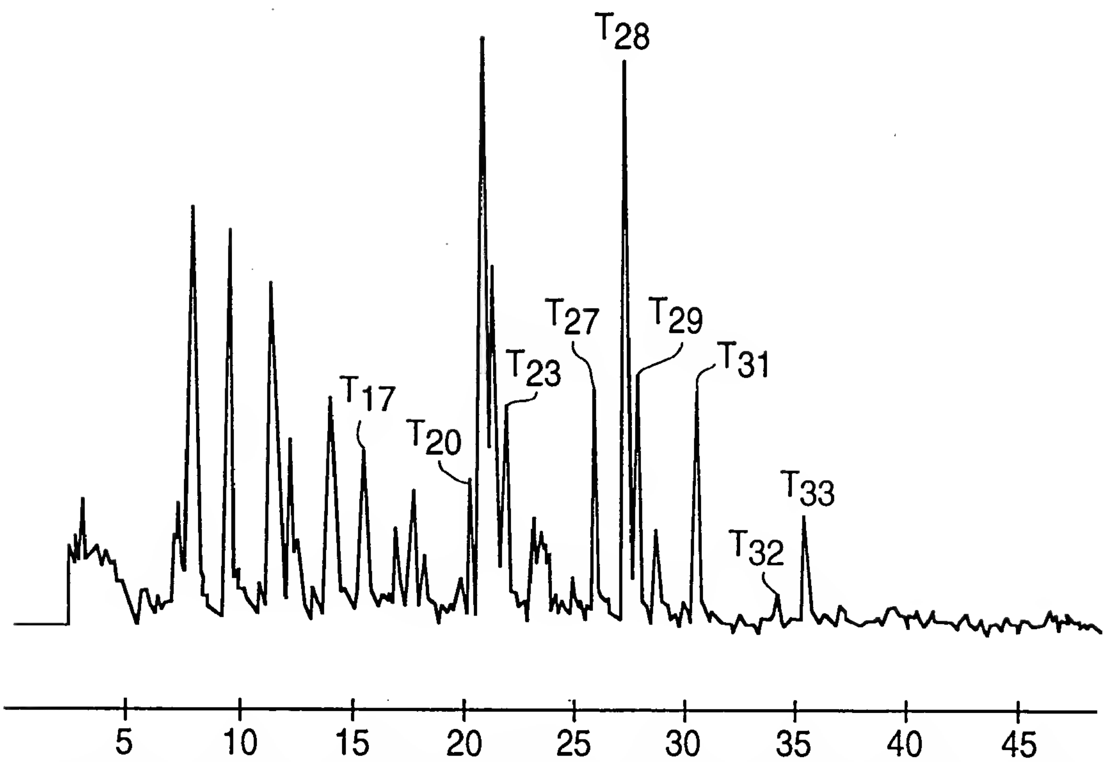
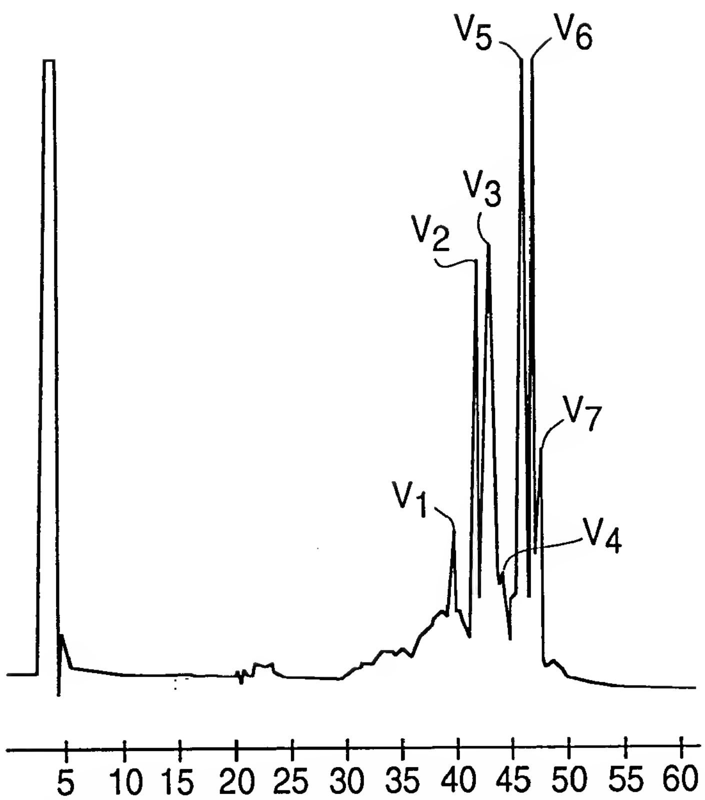
FIG. 1



ELUTION PROFILE BY MEASUREMENT OF THE OPTICAL DENSITY AT 218 nm OF THE PRODUCT OF TRYPTIC DIGESTION OF URATE OXIDASE

FIG. 2



ELUTION PROFILE BY MEASUREMENT OF THE OPTICAL DENSITY AT 218 nm
OF THE PRODUCT OF DIGESTION OF URATE OXIDASE WITH PROTEASE V8

FIG. 3

```
   1 AAACCCTCACTGCCTCTCTCATTCCTTCCG  GTGCCCCCGATCCTCAATCCAACTTGTACA 60
  61 TACTTCTCCCAACTCTCTGCTATATCCTTC  ATATTCCCATACTACAAGATGTCCGCAGTA 120
 121 AAAGCAGCCCGCTACGGCAAGGACAATGTC  CGCGTCTACAAGGTTCACAAGGACGAGAAG 180
 181 ACCGGTGTCCAGACGGTGTACGAGATGACC  GTCTGTGTGCTTCTGGAGGGTGAGATTGAG 240
 241 ACCTCTTACACCAAGGCCGACAACAGCGTC  ATTGTCGCAACCGACTCCATTAAGAACACC 300
     |
 301 ATTTACATCACCGCCAAGCAGAACCCCGTT  ACTCCTCCCGAGCTGTTCGGCTCCATCCTG 360
 361 GGCACACACTTCATTGAGAAGTACAACCAC  ATCCATGCCGCTCACGTCAACATTGTCTGC 420
 421 CACCGCTGGACCCGGATGGACATTGACGGC  AAGCCACACCCTCACTCCTTCATCCGCGAC 480
                                A*
 481 AGCGAGGAGAAGCGGAATGTGCAGGTGGAC  GTGGTCGAGGGCAAGGGCATCGATATCAAG 540
 541 TCGTCTCTGTCCGGCCTGACCGTGCTGAAG  AGCACCAACTCGCAGTTCTGGGGCTTCCTG 600
 601 CGTGACGAGTACACCACAGTTAAGGAGACC  TGGGACCGTATCCTGAGCACCGACGTCGAT 660
 661 GCCACTTGGCAGTGGAAGAATTTCAGTGGA  CTCCAGGAGGTCCGCTCGCACGTGCCTAAG 720
 721 TTCGATGCTACCTGGGCCACTGCTCGCGAG  GTCACTCTGAAGACTTTTGCTGAAGATAAC 780
 781 AGTGCCAGCGTGCAGGCCACTATGTACAAG  ATGGCAGAGCAAATCCTGGCGCGCCAGCAG 840
 841 CTGATCGAGACTGTCGAGTACTCGTTGCCT  AACAAGCACTATTTCGAAATCGACCTGAGC 900
     G*
 901 TGGCACAAGGGCCTCCAAAACACCGGCAAG  AACGCCGAGGTCTTCGCTCCTCAGTCGGAC 960
 961 CCCAACGGTCTGATCAAGTGTACCGTCGGC  CGGTCCTCTCTGAAGTCTAAATTGTAAACC 1020
1021 AACATGATTCTCACGTTCCGGAGTTTCCAA  GGCAAACTGTATATAGTCTGGGATAGGGTA 1080
1081 TAGCATTCATTCACTTGTTTTTACTTCCA  AAAAAAAAAAA...
```

NUCLEOTIDE SEQUENCE OF CLONE 9C AND OF PART OF CLONE 9A

↓ : START OF CLONE 9A

FIG. 4A

```
109 ATGTCCGCAGTAAAAGCAGCCCGCTACGGC  AAGGACAATGTCCGCGTCTACAAGGTTCAC 168
  1 MetSerAlaValLysAlaAlaArgTyrGly  LysAspAsnValArgValTyrLysValHis 20

169 AAGGACGAGAAGACCGGTGTCCAGACGGTG  TACGAGATGACCGTCTGTGTGCTTCTGGAG 228
 21 LysAspGluLysThrGlyValGlnThrVal  TyrGluMetThrVAlCysValLeuLeuGlu 40

229 GGTGAGATTGAGACCTCTTACACCAAGGCC  GACAACAGCGTCATTGTCGCAACCGACTCC 288
 41 GlyGluIleGluThrSerTyrThrLysAla  AspAsnSerValIleValAlaThrAspSer 60
                                      V3
289 ATTAAGAACACCATTTACATCACCGCCAAG  CAGAACCCCGTTACTCCTCCCGAGCTGTTC 348
 61 IleLysAsnThrIleTyrIleThrAlaLys  GlnAsnProValThrProProGluLeuPhe 80
                                          T32/T33
349 GGCTCCATCCTGGGCACACACTTCATTGAG  AAGTACAACCACATCCATGCCGCTCACGTC 408
 81 GlySerIleLeuGlyThrHisPheIleGlu  LysTyrAsnHisIleHisAlaAlaHisVal 100

409 AACATTGTCTGCCACCGCTGGACCCGGATG  GACATTGACGGCAAGCCACACCCTCACTCC 468
101 AsnIleValCysHisArgTrpThrArgMet  AspIleAspGlyLysProHisProHisSer 120

469 TTCATCCGCGACAGCGAGGAGAAGCGGAAT  GTGCAGGTGGACGTGGTCGAGGGCAAGGGC 528
121 PheIleArgAspSerGluGluLysArgAsn  ValGlnValAspValValGluGlyLysGly 140
                                     T17
529 ATCGATATCAAGTCGTCTCTGTCCGGCCTG  ACCGTGCTGAAGAGCACCAACTCGCAGTTC 588
141 IleAspIleLysSerSerLeuSerGlyLeu  ThrValLeuLysSerThrAsnSerGlnPhe 160
                                                                    T31
                V5/V6
589 TGGGGCTTCCTGCGTGACGAGTACACCACA  CTTAAGGAGACCTGGGACCGTATCCTGAGC 648
161 TrpGlyPheLeuArgAspGluTyrThrThr  LeuLysGluThrTrpAspArgIleLeuSer 180

649 ACCGACGTCGATGCCACTTGGCAGTGGAAG  AATTTCAGTGGACTCCAGGAGGTCCGCTCG 708
181 ThrAspValAspAlaThrTrpGlnTrpLys  AsnPheSerGlyLeuGlnGluValArgSer 200
                T28                           T20
```

TO FIG. 4B

TO FIG. 4B

FIG. 4B

FROM FIG. 4A — FROM FIG. 4A

```
709 CACGTGCCTAAGTTCGATGCTACCTGGGCC  ACTGCTCGCGAGGTCACTCTGAAGACTTTT 768
201 HisValProLysPheAspAlaThrTrpAla  ThrAlaArgGluValThrLeuLysThrPhe 220
                      T23

769 GCTGAAGATAACAGTGCCAGCGTGCAGGCC  ACTATGTACAAGATGGCAGAGCAAATCCTG 828
221 AlaGluAspAsnSerAlaSerValGlnAla  ThrMetTyrLysMetAlaGluGlnIleLeu 240
                 V2

829 GCGCGCCAGCAGCTGATCGAGACTGTCGAG  TACTCGTTGCCTAACAAGCACTATTTCGAA 888
241 AlaArgGlnGlnLeuIleGluThrValGlu  TyrSerLeuProAsnLysHisTyrPheGlu 260
                                               V1                T29/

889 ATCGACCTGAGCTGGCACAAGGGCCTCCAA  AACACCGGCAAGAACGCCGAGGTCTTCGCT 948
261 IleAspLeuSerTrpHisLysGlyLeuGln  AsnThrGlyLysAsnAlaGluValPheAla 280
T27

949 CCTCAGTCGGACCCCAACGGTCTGATCAAG  TGTACCGTCGGCCGGTCCTCTCTGAAGTCT 1008
281 ProGlnSerAspProAsnGlyLeuIleLys  CysThrValGlyArgSerSerLeuLysSer 300

1009 AAATTGTAA
301 LysLeuEnd
```

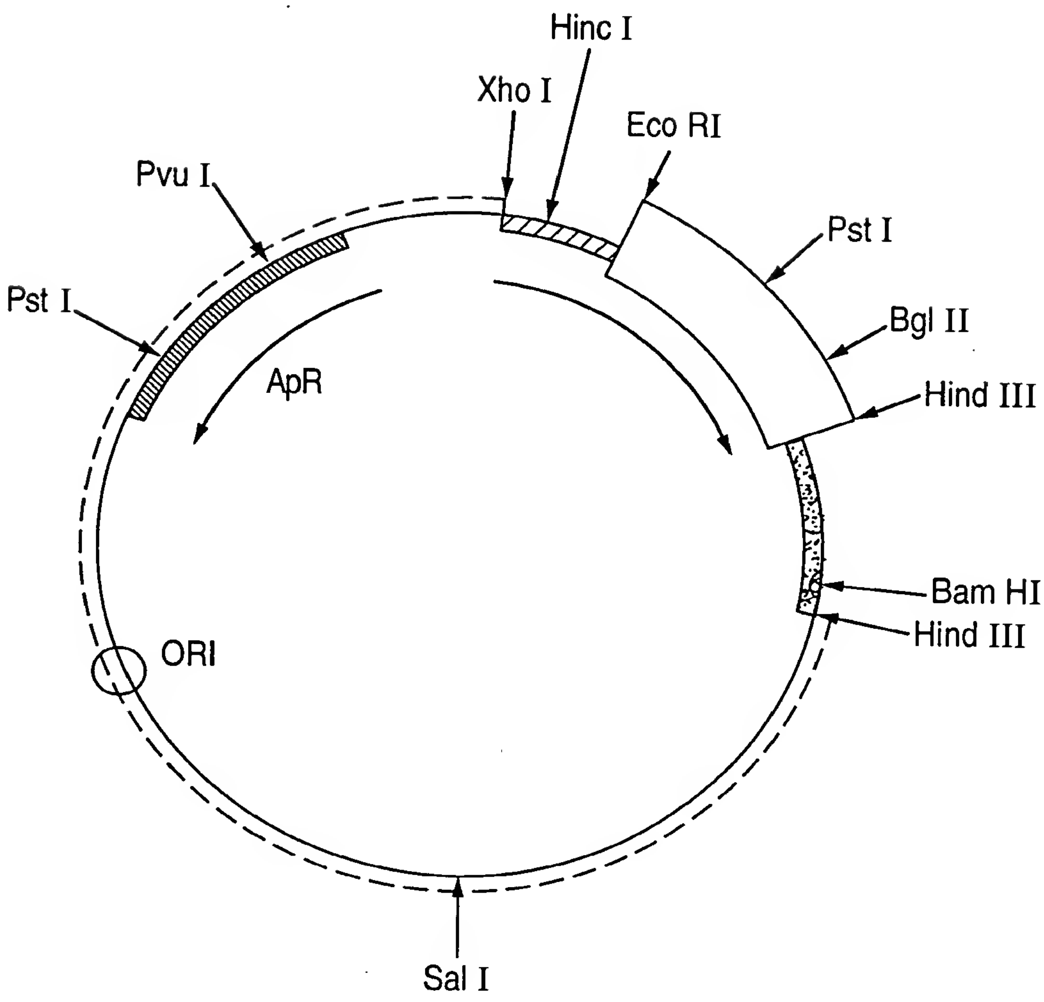
DNA SEQUENCE OPENED BY ATG IN POSITION 109 IN FIGURE 3 AND POLYPEPTIDE CODED FOR.

THE SEQUENCED PEPTIDES OBTAINED BY HYDROLYSIS OF A. FLAVUS URATE OXIDASE WITH TRYPSIN AND PROTEASE V8 ARE SHOWN BY ARROWS OPPOSITE THE POLYPEPTIDE CODED FOR, ACCORDING TO

⟷ : TRYPTIC PEPTIDE

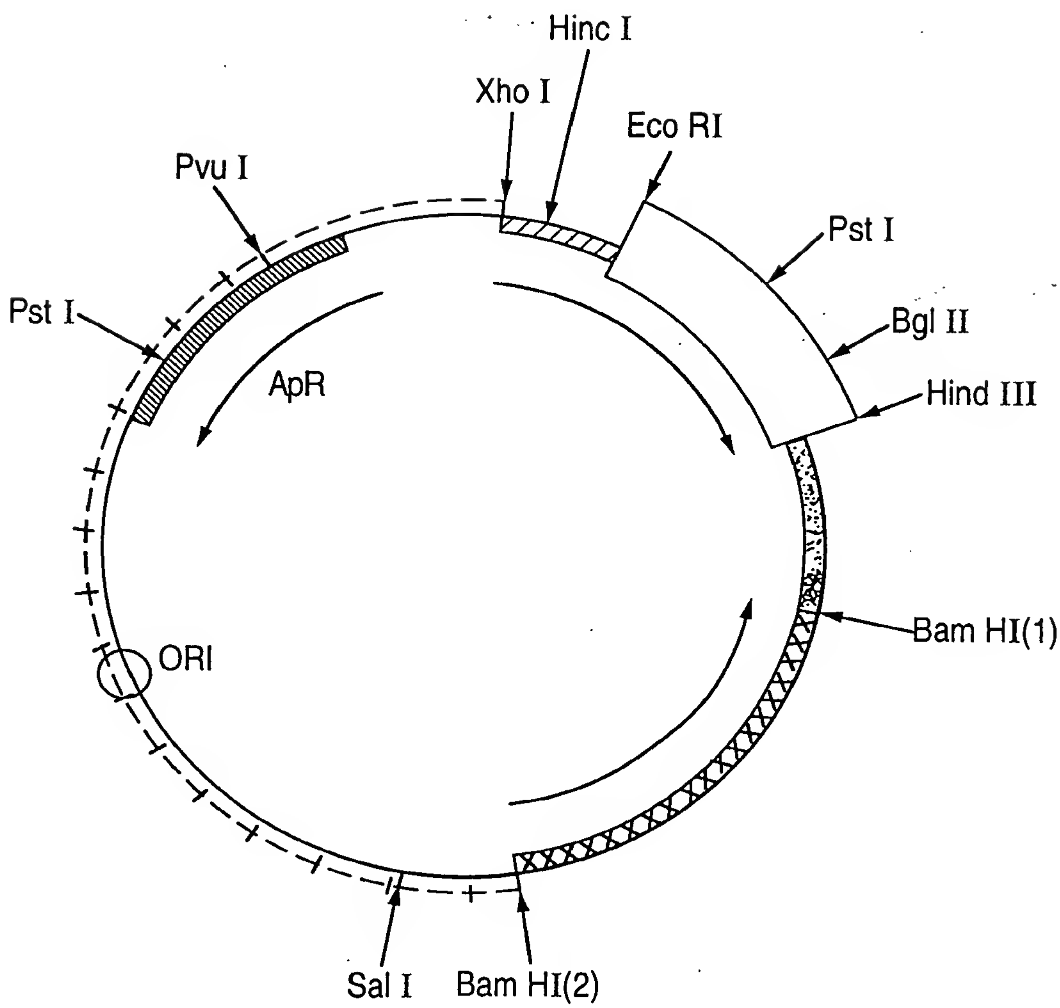
←- - -→ : PEPTIDE OBTAINED BY HYDROLYSIS WITH PROTEASE V8.

FIG. 5



Hinc I
Xho I
Eco RI
Pvu I
Pst I
Pst I
Bgl II
Hind III
ApR
Bam HI
Hind III
ORI
Sal I

FIG. 6



Hinc I
Xho I
Eco RI
Pvu I
Pst I
Pst I
Bgl II
Hind III
ApR
Bam HI(1)
ORI
Sal I
Bam HI(2)

FIG. 7

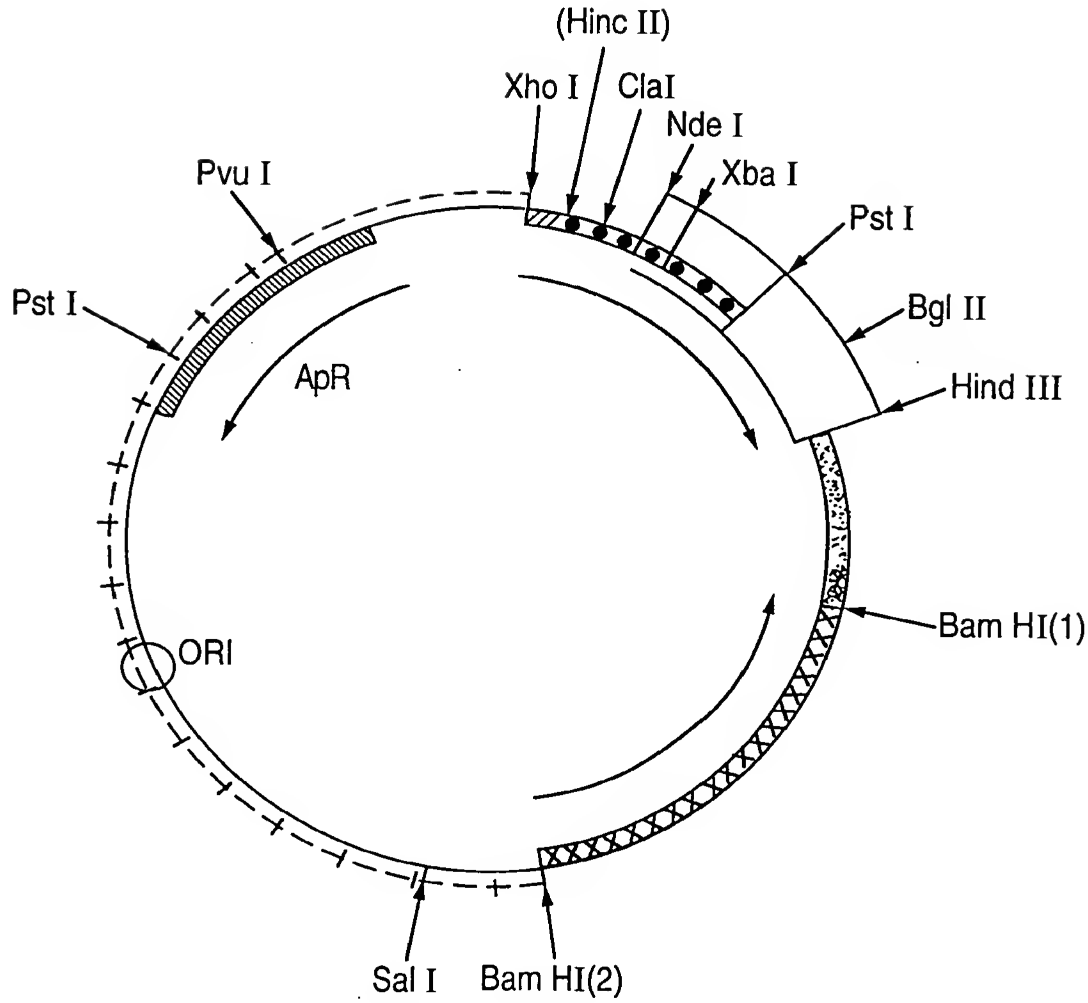
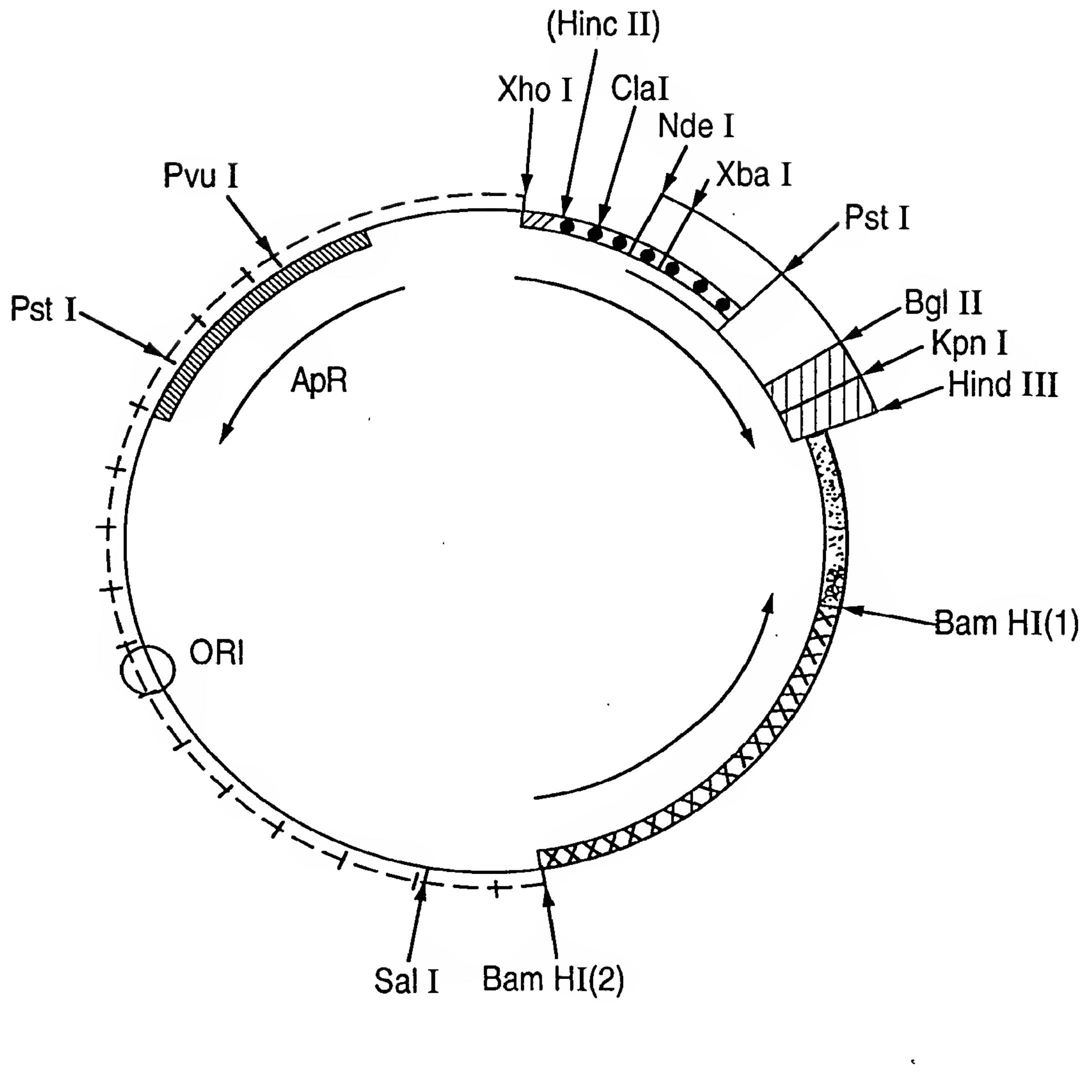
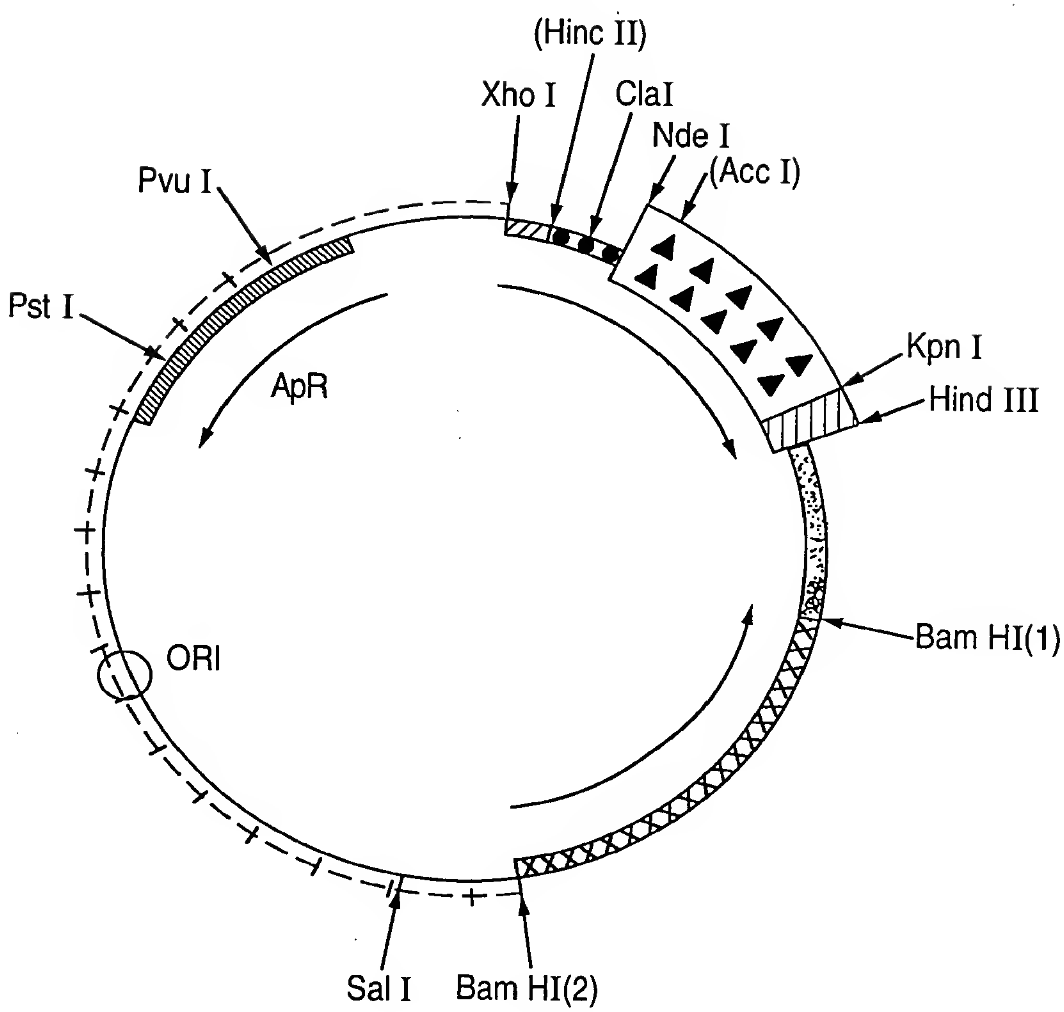

(Hinc II)
Xho I
ClaI
Nde I
Xba I
Pst I
Bgl II
Hind III
Pvu I
Pst I
ApR
ORI
Bam HI(1)
Sal I
Bam HI(2)

FIG. 8



(Hinc II)
Xho I
ClaI
Nde I
Xba I
Pst I
Pvu I
Pst I
Bgl II
Kpn I
Hind III
ApR
Bam HI(1)
ORI
Sal I
Bam HI(2)

FIG. 9


(Hinc II)
Xho I
ClaI
Nde I
(Acc I)
Pvu I
Pst I
ApR
Kpn I
Hind III
Bam HI(1)
ORI
Sal I
Bam HI(2)

10

FIG. 10

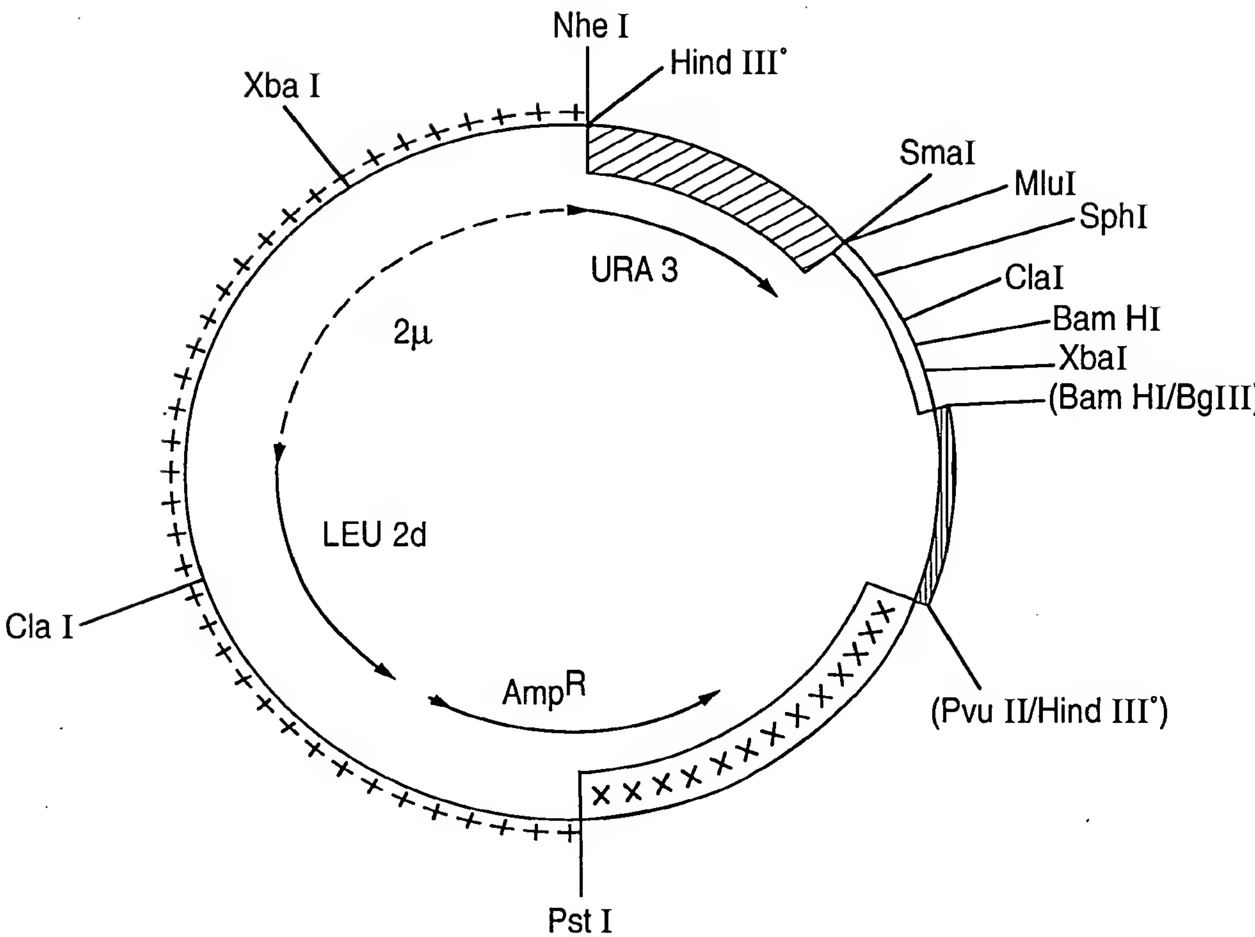
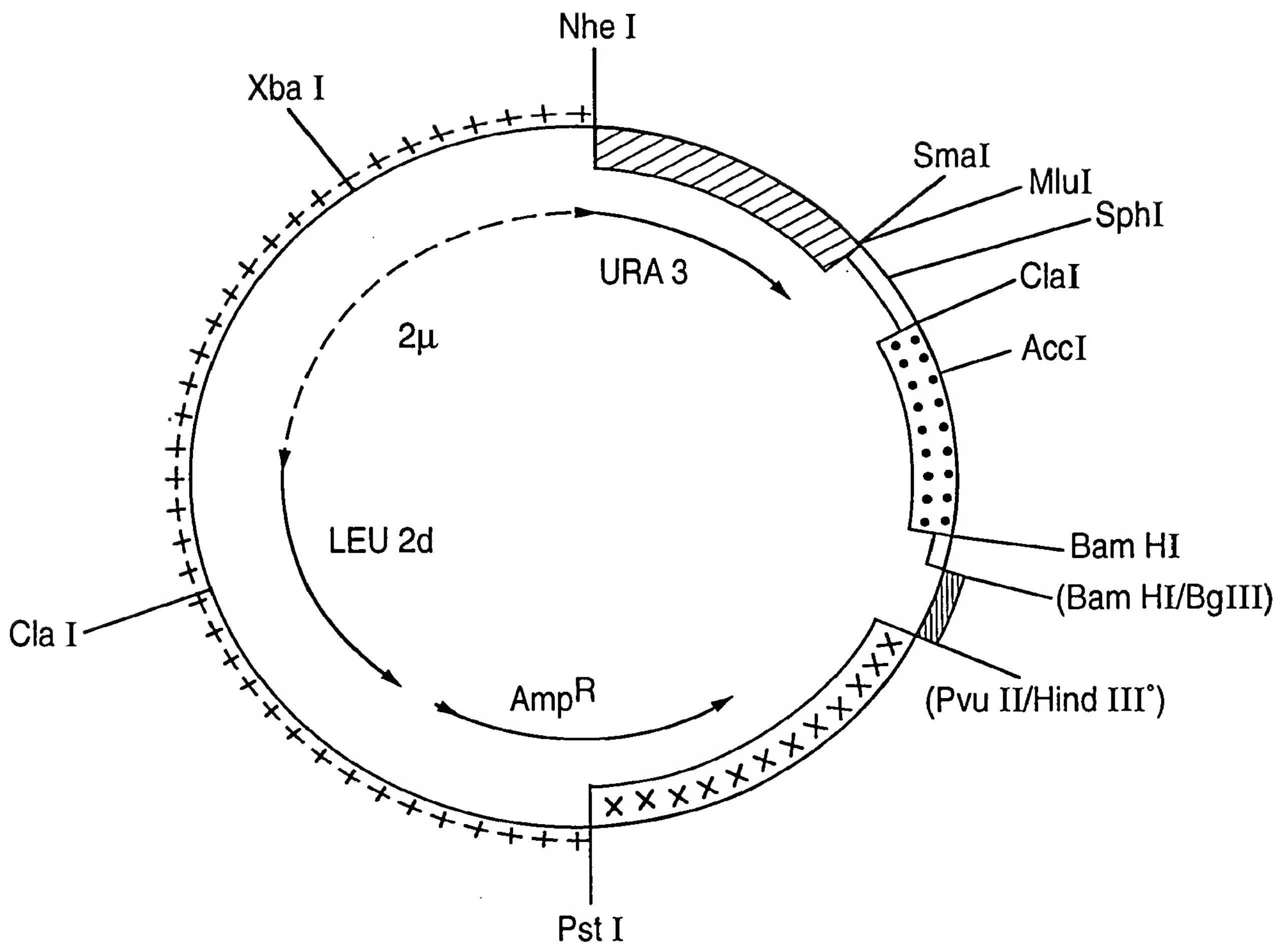
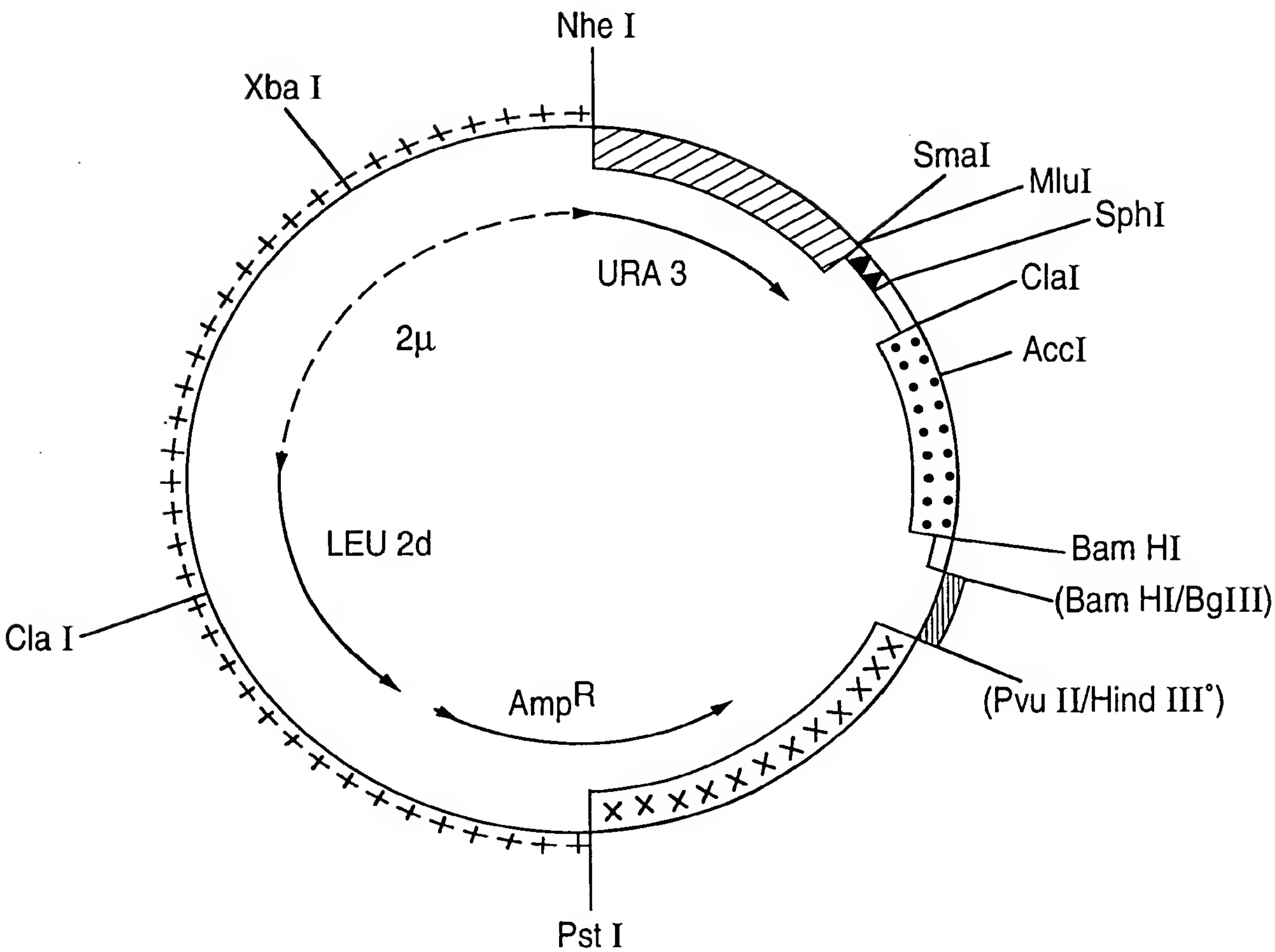

Nhe I
Hind III°
Xba I
SmaI
MluI
SphI
ClaI
Bam HI
XbaI
(Bam HI/BglII)
URA 3
2μ
LEU 2d
Cla I
AmpR
(Pvu II/Hind III°)
Pst I

FIG. 11



Nhe I
Xba I
SmaI
MluI
SphI
ClaI
AccI
URA 3
2μ
LEU 2d
Bam HI
(Bam HI/BgIII)
Cla I
AmpR
(Pvu II/Hind III°)
Pst I

FIG. 12



Nhe I
Xba I
SmaI
MluI
SphI
ClaI
AccI
Bam HI
(Bam HI/BglII)
(Pvu II/Hind III°)
Cla I
Pst I
URA 3
2μ
LEU 2d
AmpR

FIG. 13

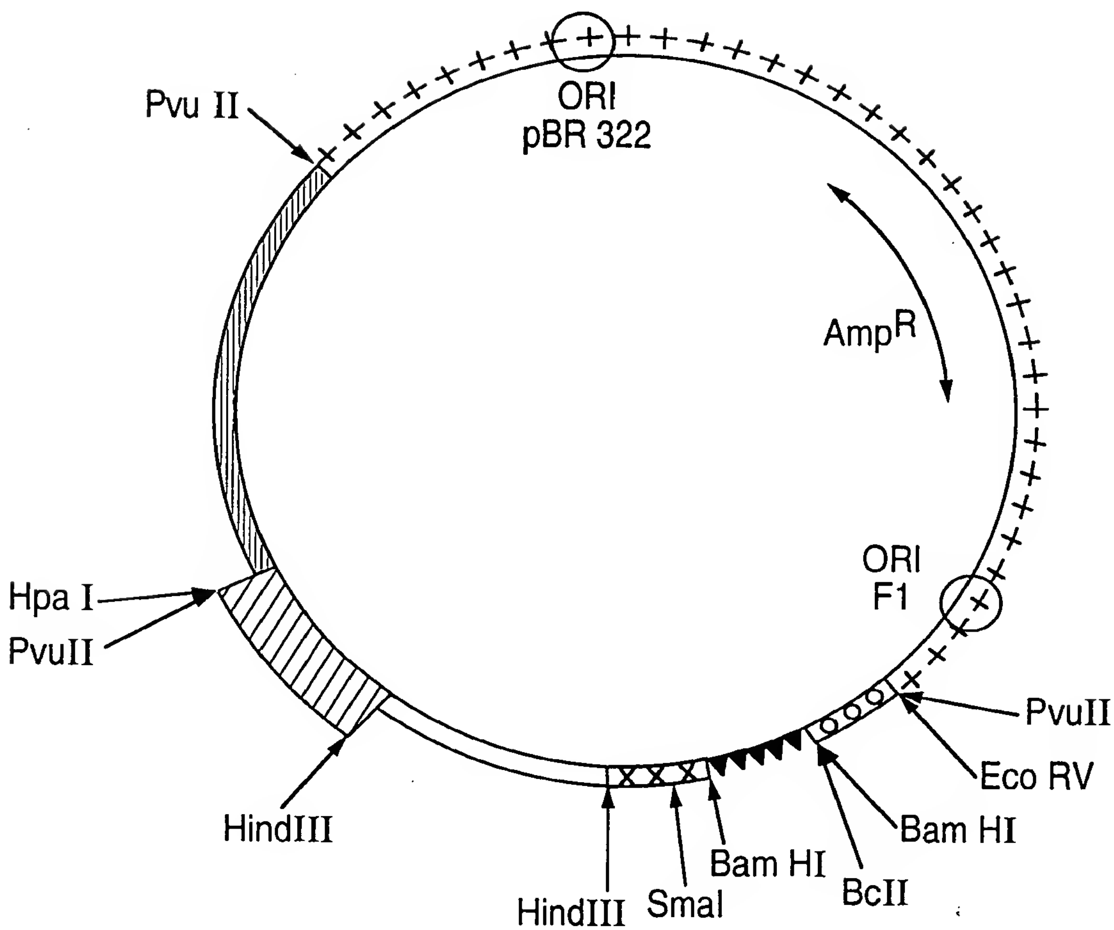
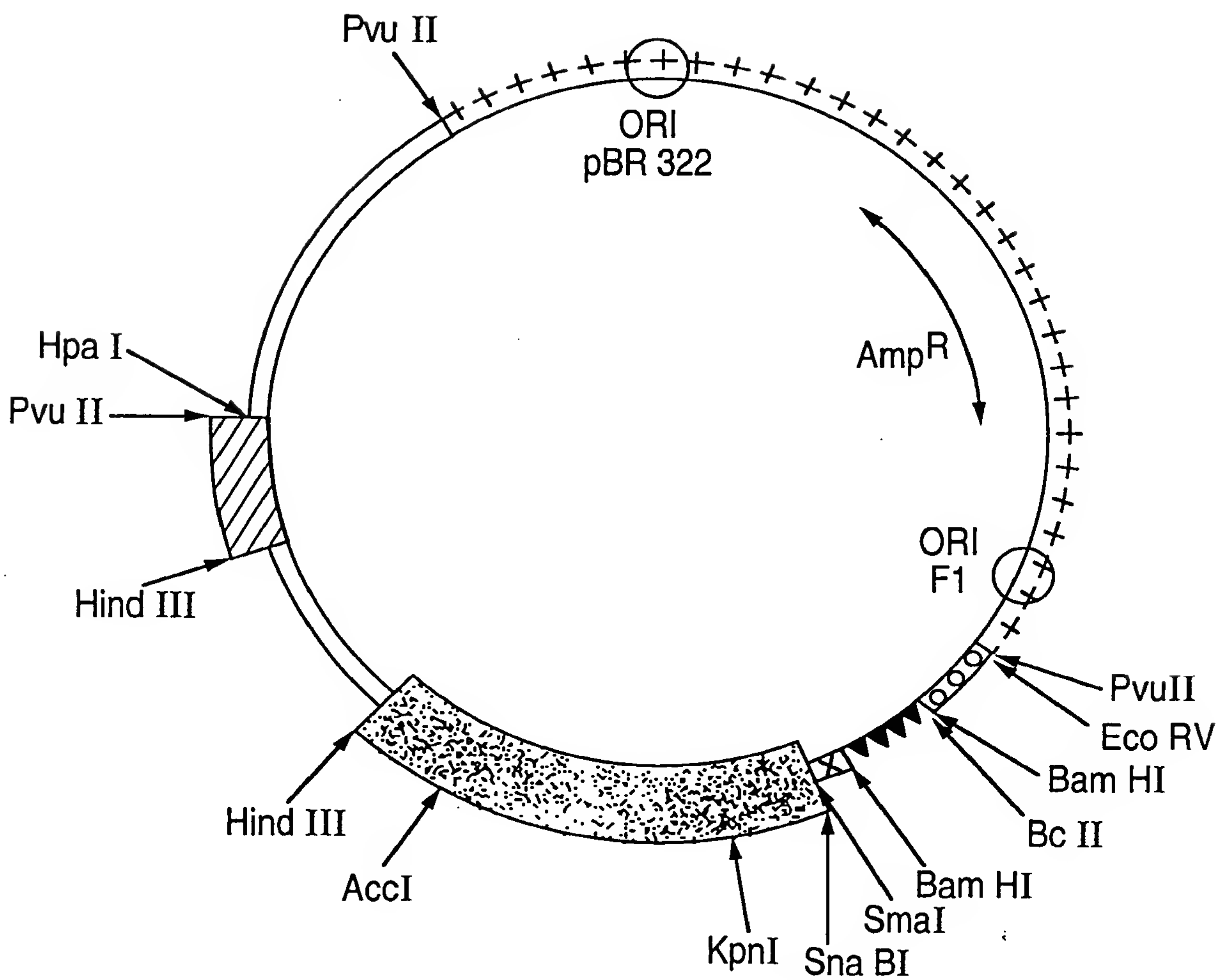

ORI
pBR 322
Pvu II
AmpR
ORI
F1
Hpa I
PvuII
PvuII
Eco RV
HindIII
Bam HI
BcII
Bam HI
HindIII
SmaI

FIG. 14



Pvu II
ORI
pBR 322
AmpR
Hpa I
Pvu II
Hind III
ORI
F1
PvuII
Eco RV
Bam HI
Bc II
Hind III
AccI
KpnI
Sna BI
SmaI
Bam HI